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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/997,504	11/19/2001	Michael E. Himmel	NREL 99-38	3921

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EXAMINER	
RAO, MANJUNATH N	
ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 10/28/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/997,504	HIMMEL ET AL.	
	Examiner	Art Unit	
	Manjunath N. Rao, Ph.D.	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the sat or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *15 August 2005*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *3,7,29 and 31* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *3,7,29 and 31* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Claims 3, 7, 29, 31 are still at issue and are present for examination.

Applicants' amendments and arguments filed on 8-15-05 have been fully considered and are deemed to be persuasive to overcome the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Priority

Applicant's claim for domestic priority under 35 U.S.C. 119(e) is acknowledged. Acknowledgment is made of applicant's claim for foreign priority based on an application filed in WIPO on 5-19-2000. It is noted, however, that applicant has not filed a certified copy of the PCT/US00/13971 application as required by 35 U.S.C. 119(b).

Examiner has received no specific response to the above from the applicant in the response filed on 8-15-05

Sequence Compliance

Applicant is required to comply with the sequence rules by inserting the correct sequence identification numbers of all sequences recited within the claims and/or specification. It is particularly noted that applicant fails to provide the sequence identification numbers for sequences recited in the amended claims 7 and 31. See particularly 37 CFR 1.821(d). Because of the absence of sequence identification numbers, Examiner was unable to do a meaningful search.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 7 and 31 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 7 and 31 recites the phrase "wherein the mutant glycosyl hydrolase is selected from the groups consisting of ...." and provides the full length sequence of 3 polynucleotides as opposed to a polypeptide sequence. It is not clear whether applicants intended to recite that the enzyme glycosyl hydrolase was encoded by a polynucleotide selected from the three. It is not clear to the Examiner as to how those skilled in the art would conclude that the polynucleotide sequence recited in the claim is that of a polypeptide. Examiner requests clarification.

Claims 29 and 31 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claims 29 and 31 are drawn to a method of increasing the specific activity of *A.cellulolyticus* E1 endoglucanase by replacing through site-directed mutagenesis an active site associated glycosyl-stabilizing tyrosine amino acid with glycine. It is not clear to the Examiner as to how those skilled in the art would know as to which specific amino acid in the full length sequence of the above enzyme should be targeted for substitution. Without the specific amino acid known, it would be meaningless for those skilled in the art to even attempt this method. Examiner requests clarification.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 3, 7, 29, 31 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of increasing the specific activity of EI endoglucanase comprising the amino acid sequence of SEQ ID NO: 10, 12, 14 (SEQ ID NO:2, 3, 4?) isolated from *Acidothermus cellulolyticus* on pretreated biomass, comprising the replacement of the specific active site associated glycosyl stabilizing amino acid, i.e., Y at position 245 with G in SEQ ID NO:10, or Y at position 42 with R in SEQ ID NO:12, or W at position 82 with R in SEQ ID NO:14 does not reasonably provide enablement for a method of increasing the specific activity of any glycosyl hydrolase enzyme isolated from any or all sources acting on any or all type of substrates, comprising the replacement of any tyrosine that is "active site associated" and "glycosyl stabilizing" with a glycine amino acid. The specification also does not reasonably provide enablement for a method of increasing the specific activity of any glycosyl hydrolase enzyme isolated from any source including any or all strains of *A.cellulolyticus* by site-directed mutagenesis such that an active site associated glycosyl-stabilizing amino acid of the endoglucanase is replaced with an amino acid, the replacing amino acid binding cellobiose less tightly than the glycosyl-stabilizing amino acid, to provide a mutant endoglucanase wherein said glycosyl-stabilizing amino acid comprises a tyrosine and the replacing amino acid comprises a glycine. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Factors to be considered in determining whether undue experimentation is required, are summarized in *In re Wands* (858 F.2d 731, 8 USPQ 2nd 1400 (Fed. Cir. 1988)) as follows: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claim(s).

Claims 3, 7, 29, 31 are so broad as to encompass a method of increasing the specific activity of any glycosyl hydrolase enzyme isolated from any or all sources acting on any or all type of substrates, comprising the replacement of any active site associated glycosyl stabilizing amino acid and a method of increasing the specific activity of any glycosyl hydrolase enzyme isolated from any or all strains of *A.cellulolyticus* by site-directed mutagenesis such that an active site associated glycosyl-stabilizing amino acid of the endoglucanase is replaced with an amino acid, the replacing amino acid binding cellobiose less tightly than the glycosyl-stabilizing amino acid, to provide a mutant endoglucanase wherein said glycosyl-stabilizing amino acid comprises a tyrosine and the replacing amino acid comprises a glycine. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the application of a single method for an extremely large number of glycosyl hydrolases broadly encompassed by the claims and with regard to the analyzing and testing of the extremely large number of mutants that result from the site-directed mutagenesis experiment on *A.cellulolyticus* endoglucanase. Since the amino acid sequence of a protein determines its structural and functional properties, predictability of which changes can be tolerated in a protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to

which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e. expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. However, in this case the disclosure is limited to the single method of making a variant by changing the amino acid Y at position 245 to G in SEQ ID NO:10, or Y at position 42 to R in SEQ ID NO:12, or W at position 82 to R in SEQ ID NO:14. It would require undue experimentation of the skilled artisan to make and use the claimed method to increase the specific activity of any or all glycosyl hydrolases including structural analogs of endoglucanases. The specification is also limited to the single method of enhancing the specific activity of a single EI endoglucanase against a single substrate, i.e., pretreated biomass, but provides no guidance with regard to using the same method on any glycosylhydrolase isolated from any source and having any structure or with regard to the analysis of the extremely large number of mutants and variants that arise from the site-directed mutation of *A.cellulolyticus* endoglucanase. In view of the great breadth of the claim, amount of experimentation required to use the above method on any glycosyl hydrolase which includes a large number of different hydrolytic enzymes acting on a wide range of substrates, the lack of guidance, working examples, and unpredictability of the art in predicting function from a polypeptide primary structure (e.g., see Ngo et al. in The Protein Folding Problem and Tertiary Structure Prediction, 1994, Merz et al. (ed.), Birkhauser, Boston, MA, pp. 433 and 492-495, Ref: U, Form-892), the claimed invention would require undue experimentation. As such, the specification fails to teach one of ordinary skill how to use the full scope of the method encompassed by these claims.

While recombinant and mutagenesis techniques are known, and it is routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions.

The specification does not support the broad scope of the claims which encompasses a method of increasing the specific activity of any glycosyl hydrolase enzyme isolated from any or all sources acting on any or all type of substrates, comprising the replacement of any active site associated glycosyl stabilizing amino acid because the specification does not establish that: (A)the method works in all or any glycosyl hydrolases which includes a large number of different enzymes; (B) the specific activity of the glycosyl hydrolases is enhanced irrespective of the type of substrate; (C) a rational and predictable scheme for identifying and modifying any active site residue in any glycosyl hydrolase with an expectation of obtaining the desired biological function; and (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

Thus, applicants have not provided sufficient guidance to enable one of ordinary skill in the art to use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including all or any glycosyl hydrolase and all or any type of substrates. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of a

method that applies to all types of glycosyl hydrolases and all types of substrates is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

In response to the previous Office action, applicant argues, in view of the amendments made to these claims and the fact that the claims should be read with reference to the specification commencing at page 6, line 16 through page 7, line 16, together with the results in Tables 1-4 (which make abundantly clear, the method of increasing the specific activity of the E1 endoglucanase comprising the mentioned amino acid sequences), the rejection is no longer applicable. Examiner respectfully disagrees. While it is agreed that the claims should be read in light of the specification, to conclude that said claims are also limited to the teachings in the specification is highly misplaced conclusion. In this case, while Examiner agrees that applicant has support for specific examples and the specification is enabled for a method of making of those specific analogs, he respectfully disagrees that such guidance is enough for claiming a method of increasing the specific activity of any glycosylhydrolases as claimed herein. It must be remembered that the family of glycosylhydrolases includes a variety of hydrolases which have separate class of substrates and results in generation of a variety of products. The examples provided by the applicant is specific for endoglucanases only but not all members of glycosyl family. As stated in earlier Office actions, applicant's arguments are not persuasive because while methods to produce variants of a known sequence such as site-specific mutagenesis, random mutagenesis, etc. are well known to the skilled artisan, method of increasing the specific activity of all members of glycosyl hydrolases as claimed by applicants requires that one of ordinary skill in the art know or be provided with a universal method for the selection of

specific amino acid residues in each and every member of the glycosyl hydrolase family and/or provide a single universal method to identity those specific mutants and variants that arise from site-directed mutagenesis experiments to be comprised with a substituted glycosyl-stabilizing amino acid as claimed in claim 29. Without such guidance one of ordinary skill would be reduced to the necessity of producing and testing all of the virtually infinite possibilities. This would clearly constitute undue experimentation. While enablement is not precluded by the necessity for routine screening, if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Such guidance has not been provided in the instant specification. Hence the above rejection is maintained.

Claim 3 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim 3 is directed to a method of enhancing the specific activity of any glycosyl hydrolase. Claim 3 is rejected under this section of 35 USC 112 because the claim is directed to a method of using a genus of polypeptides that have not been disclosed in the specification. No description has been provided of the polypeptide sequences encompassed by the claim. No information, beyond the characterization of a single specific polypeptide having EI-endoglucanase activity and isolated from *A.cellulolyticus* (in Example 7) has been provided by applicants which would indicate that they had possession of the claimed genus of polypeptides.

The specification does not contain any disclosure of the structure of the polypeptide sequences within the scope of the genus to be used in the claimed method. The genus of polypeptides is a large variable genus including peptides which can have a wide variety of structures. Therefore many structurally unrelated polypeptides are encompassed within the scope of these claims. The specification discloses a method for only a single species of the genus which is insufficient to put one of skill in the art in possession of the attributes and features of all species within the genus for use in the claimed method. Therefore, one skilled in the art cannot reasonably conclude that applicant had possession of the claimed invention at the time the instant application was filed.

Applicant is referred to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, published in the Official Gazette and also available at www.uspto.gov.

In response to the previous Office action, applicants argue claim 3, as presently amended, in fact recites the method of enhancing a specific activity of any glycosyl hydrolase because - as mentioned on page 2 of the specification between lines 15 and 29, in these cellulase enzymes there are 21 families of catalytic domains, each classified on the basis of similarity of their amino acid sequences and the location where glycine may be substituted by tryptophan, or where glycine, alanine, valine, serine, etc. may be substituted (page 7, lines 1-5 - as well as Table 1) and page 7, lines 13-16 (where it is stated that 3 or 4 mutations were made for each E1 site that included Ala, Gly, Glu and Arg), as a process for making and using these enzymes using various mutagenesis kits for site directed mutagenesis (SDM) is unquestionably clear. Examiner respectfully disagrees with the above argument. It can be readily seen that applicants are trying to include the specification as claim limitation even though the claim is drawn to a much

broader invention. While it is agreed that the claims should be read in light of the specification, to conclude that said claims are also limited to the teachings in the specification is highly misplaced conclusion.

As discussed in the written description guidelines, the written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. A representative number of species means that the species which are adequately described are representative of the entire genus. **Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.** Satisfactory disclosure of a representative number depends on whether one of skill in the art would recognize that the applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed. For inventions in an unpredictable art, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only one species within the genus. In the instant case the claimed method comprises the use of genera which includes species that are widely variant in function. The genus of polypeptides for use in the claimed method is structurally diverse. As such, the description of solely the function of polypeptide (i.e., glycosylhydrolase) present in all members of the genus is not sufficient to be representative of the attributes and features of the entire genus.

Conclusion

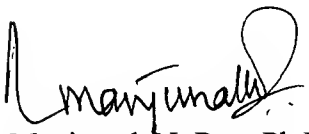
None of the claims are allowable.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the Examiner should be directed to Manjunath N. Rao, Ph.D. whose telephone number is 571-272-0939. The Examiner can normally be reached on 7.00 a.m. to 3.30 p.m. If attempts to reach the examiner by telephone are unsuccessful, the Examiner's supervisor, Ponnathapura Achutamurthy can be reached on 571-272-0928. The fax phone numbers for the organization where this application or proceeding is assigned is 571-273-8300 for regular communications and for After Final communications. Any inquiry of a general nature or relating to the status of

this application or proceeding should be directed to the receptionist whose telephone number is 571-272-1600.



Manjunath N. Rao, Ph.D.
Primary Examiner
Art Unit 1652

October 24, 2005